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EXAMINER	
LANDSMAN, ROBERT S	
ART UNIT	**PAPER NUMBER**
1647	7

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/679,852
	Examiner Robert Landsman

-- The MAILING DATE of this communication appears on the cover sheet with the [illegible] ALL J.

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FRO[illegible] -5 --
THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered time[illegible]
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this com[illegible]
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 February 2002*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) *21-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☑ disapproved by the Examiner.

If approved, corrected drawings are required in reply[illegible]

12)☐ The oath or declaration is objected to[illegible]

Priority under 35 U.S.C. §§ 119[illegible] claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

[illegible]☐ None of:

13)☐ Acknowledgm[illegible]d copies of the priority documents have been received.

a)☐ All[illegible] certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 3.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Formal Matters

A. The Response to the Restriction Requirement, filed 2/20/02, has been entered into the record.

B. Claims 1-26 are pending in the application and were subject to restriction. In the response to the Restriction Requirement of Paper No. 5, Applicants elected Group I, claims 1-5, with traverse, and argued that no serious search burden would be required to examine all of the claims, especially given that the claims of Groups I and II are in the same class and subclass. Since a method of determining whether a compound modulates G protein-coupled receptors by altering their ability to oligomerize would not require a serious burden of search over that of searching a method of detecting this oligomerization, the Examiner will examine the claims of Group I (claims 1-5) and Group II (6-20) together. However, not only would searching the fusion protein of Group III require a separate search from the methods of using this protein, but the fusions protein of Group III and the methods of using this protein (i.e. Groups I and II) are related as a product and process of use. The fusion protein of Group III can be used for purposes other than for methods of detecting oligomerization, such as in the production of antibodies. Therefore, the claims of Group III will not be recombined with Groups I and II and will, therefore, not be examined. However, the Examiner does not feel that searching for methods of detecting oligomerization wherein the first and second G protein-coupled receptor regions are the "same" or "different" is an undue search burden. Therefore, the species election required on page 3 of the Restriction Requirement of Paper No. 5 has been withdrawn and claims 1-20 will be searched in their entirety. This restriction is deemed proper and is, therefore, made FINAL.

AdK 5/7/02

2. Specification

A. The specification is objected to since the title of the invention is not descriptive. The title recites "G protein coupled receptors function as oligomers in vivo." However, the claims are drawn toward methods of detecting oligomerization of G protein-coupled receptors as well as using and screening modulators, or for potential modulators, thereof. Therefore, a new title is required that is clearly indicative of the invention to which the claims are directed. The following title, for example, is suggested: "Methods of detecting and modulating oligomerization of G protein-coupled receptors."

B. The specification is further objected to since the Brief Description of the Figures does not provide a brief description of Figure 4. Similarly, The Brief Description of the Figures also discusses Figure 3c, which is not present in the Figures themselves. It appears from the Brief Description of the Figures that Figure 4 should actually be labeled as Figure 3c. This would then be consistent with the Brief Description of the Figures which discusses parts a-c of Figure 3 and does not discuss Figure 4. Further evidence that Figure 4 should be renumbered as Figure 3c is due to the fact that there is a "c" on the page labeled "Figure 4," implying that this is actually panel "c" of Figure 3.

C. The specification is further objected to since references 8 and 10 are missing from the "References" section on page 29 of the disclosure.

3. Claim Rejections - 35 USC § 112, first paragraph - enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

A. Claims 11-20 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

To understand this rejection, claim 6 needs to be understood. Claim 6 recites that it can be determined whether an agonist activates G protein-coupled receptors (GPCRs) by either enhancing or disrupting oligomerization since, if the efficiency of FRET detected in cells contacted with an agonist is *greater* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by *enhancing* oligomerization. Conversely, if the efficiency of FRET detected in cells contacted with an agonist is *less* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by *disrupting* oligomerization.

According to claim 6, the artisan is *certain* that the compound used in the method of this claim is, in fact, an agonist, and simply want to further elucidate its mechanism of action with regard to oligomerization of GPCRs. However, this claim reveals that an agonist can activate G protein-coupled receptors by either enhancing or disrupting receptor oligomerization. Given this, the artisan, in screening for an unknown agonist or antagonist by performing the method of either claim 11 or 16 would notice one of three possible effects on FRET efficiency upon contacting the cells of the claim with the test

compound: the FRET efficiency in cells contacted with the test compound would either increase (i.e. greater FRET efficiency), decrease (i.e. less FRET efficiency), or remain the same relative to the FRET efficiency in said cells prior to contact with said test compound.

According to claim 11, an increase in FRET efficiency in cells contacted with the test compound relative to the FRET efficiency in said cells prior to contact with said test compound would reveal that the test compound is an agonist, whereas, according to claim 16, a decrease in FRET efficiency in cells contacted with the test compound relative to the FRET efficiency in said cells prior to contact with said test compound would reveal that the test compound is an antagonist. However, taking into account that, according to claim 6, and page 2, lines 10-23 of the specification, FRET efficiency in cells in the presence of a known agonist could either increase or decrease FRET efficiency relative to that seen prior to the addition of the agonist, the artisan, upon observing either said increase or decrease in FRET efficiency, would not be able to determine whether the test compound of claim 11 and claim 16 was an agonist or an antagonist of the interaction of GPCR oligomerization, since, according to claim 6, agonists themselves produce the effects recited in both claims 11 and 16.

Again, as can be deduced from reading claim 6, and page 2, lines 10-23 of the specification, even if the artisan observed a decrease in FRET efficiency in the method if claim 11, the artisan would not be able to conclude that the test compound was *not* an agonist, since, according to claim 6, and page 2, lines 10-23 of the specification, agonists have the ability to decrease, as well as increase, FRET efficiency. For these same reasons, if the artisan observed a decrease in FRET efficiency in the method of claim 16, the artisan would not be able to conclude that the test compound was an antagonist.

Claim 11 recites that it can be determined that a test compound is an agonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is greater than the FRET efficiency in said cells prior to contact with said test compound. This would, according to claim 6 and page 2, lines 10-23 of the specification, reveal that the agonist was acting by enhancing oligomerization of the G protein-coupled receptors. However, while it is understood how a test compound could be considered an agonist by enhancing oligomerization of GPCRs, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt oligomerization of GPCRs. This would be determined if the efficiency of FRET detected in cells contacted with an agonist is *less* than that detected in cells prior to contact with said agonist. However, it would not be clear to the artisan performing the method of claim 11 that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound was not still, in fact, an agonist,

since, again, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt (i.e. decreased or "less" FRET) oligomerization of GPCRs.

Claim 16 recites that it can be determined that a test compound is an antagonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. However, according to claim 6 and page 2, lines 10-23 of the specification, *agonists* can also act by disrupting oligomerization of the G protein-coupled receptors if the efficiency of FRET detected in cells contacted with an agonist is less than that detected in cells prior to contact with said agonist. Therefore, the method of claim 16 would not enable the artisan to identify an antagonist of GPCR interaction since it would not be clear to the artisan performing this method that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound is, in fact, an antagonist, since, according to claims 6 and page 2, lines 10-23 of the specification, the same criteria are used to identify an agonist. Claims 12-15 and 17-20 are rejected since they depend from rejected base claims.

B. Claims 16-20 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 16 recites that a test compound can be identified as an antagonist of the interaction between GPCRs if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. The only logical way to interpret the method of claim 16 is that the antagonist would be reversing GPCR oligomerization, since this compound would be, according to the claim, *decreasing* FRET efficiency. This implies that the receptors were initially oligomerized and that FRET was detected in the absence of the test compound (antagonist).

However, it is well-known to one of ordinary skill in the art that antagonists, by definition, do not produce any effect on a receptor. The only "function" of an antagonist is to bind to a receptor to prevent, or reverse, the effects of an agonist, which does produce an effect on a receptor (Gilman AG. et al., The Pharmacological Basis of Therapeutics, 1993 - page 33, right column, second paragraph and page 45, right column, first paragraph). Therefore, based on this definition, a test compound which has the effect of decreasing FRET efficiency in a system is not an antagonist, but, in fact, an agonist. The only way that the test compound identified in claim 16 could truly be considered an antagonist is if the claimed cells containing these GPCRs already contained an endogenous agonist for these GPCRs which allowed the

initial oligomerization of these GPCRs. In this case, the artisan would observe an increase in FRET efficiency in the absence of a test compound due to the endogenous GPCR agonist in the cells. Therefore, any test compound which *then* decreased this agonist-induced FRET efficiency could then be considered an antagonist, since it would be competing with the agonist in the system to nullify the effect of said agonist and *not* producing any effect on its own. However, the claims as written do not enable the artisan to determine that the test compound is an antagonist. Claims 17-20 are rejected since they depend from rejected base claims.

4. Claim Rejections - 35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A. Claims 1-20 are confusing since independent claims 1, 6, 11 and 16 recite "transfecting a cell with G protein coupled receptor fusion proteins." However, cells are transfected with nucleic acid molecules encoding these fusion proteins and not with the fusion proteins themselves (see page 8, line 14 – page 9, line 15 and the Example on page 22, line 18 – page 24, line 16 of the specification). Furthermore, the term "obtaining" does not add to the meaning of the claim since it is understood that in order to "transfect" a cell with (nucleic acid encoding) G protein coupled receptor fusion proteins, the artisan would need to have already obtained these fusion proteins, or DNA encoding these fusion proteins. Therefore, parts (a) – (c) of claims 1, 6, 11 and 16 could be reworded, for example, as follows:

a) transfecting a cell with:
   i) a polynucleotide encoding a first G protein-coupled receptor containing a fluorescence donor, and;
   ii) a polynucleotide encoding a second G protein-coupled receptor containing a fluorescence acceptor;
b) exciting the fluorescence donor…

Claims 2-5, 7-10, 12-15 and 17-20 are rejected since they depend from rejected claims.

B. Claims 2, 3, 5, 7, 8, 10, 12, 13, 15, 17, 18 and 20 are confusing since it is not clear as to what "receptor regions" the claims are referring. Furthermore, in claims 2, 3, 7, 8, 12, 13, 17 and 18, it is not understood what is meant by the recitation of the receptor regions of the fusion protein being the "same" or "different." Again, it is not clear as to what "receptor regions" the claims are referring. If the claims are intended to differentiate between homodimers (i.e. "same") and heterodimers (i.e. "different"), as discussed on page 7, lines 4-8 of the specification, then the term "region" should be removed and the claims should be amended to recite that the G protein-coupled receptors are either "homologous," (claims 2, 7, 12 and 17) or "heterologous" (claims 3, 8, 13 and 18). It is suggested that the term "regions" be removed from the claims.

C. Claims 5, 10, 15 and 20 are confusing since it is not clear which fusion protein is to be truncated, since there are two fusion proteins of the invention.

D. Claims 11-15 are confusing since part (f) of claim 11 recites that it can be determined that a test compound is an agonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is greater than the FRET efficiency in said cells prior to contact with said test compound. This would, according to claim 6 and page 2, lines 10-23 of the specification, reveal that the agonist was acting by enhancing oligomerization of the G protein-coupled receptors. However, while it is understood how a test compound could be considered an agonist by enhancing oligomerization of GPCRs, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt oligomerization of GPCRs. This would be determined if the efficiency of FRET detected in cells contacted with an agonist is *less* than that detected in cells prior to contact with said agonist. However, it would not be clear to the artisan performing the method of claim 11 that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound was not still, in fact, an agonist, since, again, according to claim 6 and page 2, lines 10-23 of the specification, an agonist could also disrupt (i.e. decreased or "less" FRET) oligomerization of GPCRs. Claims 12-15 are rejected since they depend from rejected claim 11.

E. Claims 16-20 are confusing since part (f) of claim 16 recites that it can be determined that a test compound is an antagonist of GPCR interaction if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. However, according to claim 6 and page 2, lines 10-23 of the specification, *agonists* of GPCR interaction

can also act by disrupting oligomerization of the G protein-coupled receptors, since the efficiency of FRET detected in cells contacted with an agonist would also be less than that detected in cells prior to contact with said agonist. Therefore, it would not be clear to the artisan performing the method of claim 16 that, if the efficiency of FRET detected in cells contacted with a test compound is less than that detected in cells prior to contact with said test compound, that said test compound which, according to the method of claim 16 would identify an antagonist, was not still, in fact, an agonist. Claims 17-20 are rejected since they depend from rejected claim 16.

F. Claims 16-20 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Claim 16 recites that a test compound can be identified as an antagonist of the interaction between GPCRs if the FRET efficiency in cells contacted with the test compound is less than the FRET efficiency in said cells prior to contact with said test compound. The only logical way to interpret the method of claim 16 is that the antagonist would be reversing GPCR oligomerization, since this compound would be, according to the claim, *decreasing* FRET efficiency. This implies that the receptors were initially oligomerized and that FRET was detected in the absence of the test compound (antagonist).

However, it is well-known to one of ordinary skill in the art that antagonists, by definition, do not produce any effect on a receptor. The only "function" of an antagonist is to bind to a receptor to prevent, or reverse, the effects of an agonist, which does produce an effect on a receptor (Gilman AG. et al., The Pharmacological Basis of Therapeutics, 1993 - page 33, right column, second paragraph and page 45, right column, first paragraph). Therefore, based on this definition, a test compound which has the effect of decreasing FRET efficiency in a system is not an antagonist, but, in fact, an agonist. The only way that the test compound identified in claim 16 could truly be considered an antagonist is if the claimed cells containing these GPCRs already contained an agonist for these GPCRs which allowed the initial oligomerization of these GPCRs (i.e. endogenous ligand). In this case, the artisan would observe an increase in FRET efficiency in the absence of a test compound due to the endogenous GPCR agonist in the cells. Therefore, any test compound which *then* decreased this agonist-induced FRET efficiency could then be considered an antagonist, since it would be competing with the agonist in the system to nullify the effect of said agonist and *not* producing any effect on its own. If the test compound decreased FRET efficiency in the absence of an agonist endogenous to the cells used in claim 16, then that is evidence that the GPCRs of the invention must oligomerize spontaneously in order to produce FRET initially. In other words, there is constitutive oligomerization of these GPCRs. In this case, the test compound would not be

an antagonist, but an agonist since it is producing an effect on a receptor. Therefore, claim 16 lacks the essential step of determining whether or not endogenous agonists to these GPCRs exist in the cell. Claims 17-20 are rejected since they depend from rejected claim 16.

5. Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

A. Claims 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over White et al. (reference AG on the IDS of Paper No.3) in view of Miyawaki et al. (Nature 388:882-887, 1997) and further in view of Hebert et al. (reference AI on the IDS of Paper No. 3). Claim 1 recites a method of detecting oligomerization of GPCRs by transfecting a cell with a GPCR fused to a fluorescence donor and a GPCR fused to a fluorescence acceptor, exciting the fluorescence donor and detecting FRET such that if the FRET emission is greater that the FRET emission in control cells expressing only the acceptor, then GPCR oligomerization is detected. Claim 6 recites a method of determining whether an agonist enhances or disrupts GPCR oligomerization by transfecting a cell with a GPCR fused to a fluorescence donor and a GPCR fused to a fluorescence acceptor, exciting the fluorescence donor and detecting FRET such that, if the efficiency of FRET detected in cells contacted with an agonist is *greater* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by enhancing oligomerization. Conversely, if the efficiency of FRET detected in cells contacted with an agonist is *less* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by disrupting oligomerization. Claims 2 and 7 recite that, in their respective methods, the GPCR regions are the same. Claims 3 and 8 recite that, in their respective methods, the GPCR regions are different. Claims 4 and 9 recite that, in their respective methods, the fluorescence donor is cyan fluorescent protein and the fluorescence acceptor is yellow fluorescent protein.

White et al. teach that homodimerization has been reported for several seven transmembrane receptors (i.e. GPCRs), including the δ-opioid, β2-adrenergic and metabotropic glutamate receptors (page 682, left column, first paragraph). White et al. also teach that G protein-coupled $GABA_BR2$ (R2) receptors form heterodimers with G protein-coupled $GABA_BR1$ (R1) receptors (Abstract and Figure 2b). Therefore, White et al. teach that both homodimerization and heterodimerization of GPCRs does occur

and that this oligomerization (protein-protein interaction) is required for receptor activity. This can be seen in Figure 2a on page 680 of White et al. where neither R1, nor R2 are able to increase β-galactosidase activity either separately with only empty vector controls, or when attempting to be expressed as homodimers, but only when R1 and R2 are co-expressed. White et al. also teach that the known receptor agonist, GABA, is able to stimulate [^{35}S]GTPγS binding in cells expressing both R2 and R1 receptors, but not in cells expressing only one of these receptors (Figure 4b). This experiment provides further evidence that $GABA_B$ receptors oligomerize (dimerize). However, White et al. do not teach the use of FRET to detect this oligomerization, regardless of whether or not the GPCR regions are the same, or different, or whether the receptor region of the GPCR fusion protein is truncated, nor do they teach the use of cyan fluorescence protein or yellow fluorescence protein. Furthermore, White et al. do not teach a method of determining whether a receptor agonist activates GPCRs by enhancing or disrupting oligomerization of these GPCRs.

However, Miyawaki et al. do teach the use of fusing the fluorescence indicators cyan fluorescence protein (CFP) and yellow fluorescence protein (YFP) to the non-GPCR calmodulin and M13 proteins, respectively (page 887 under "Gene construction"), in order to monitor protein heterodimerization, as well as to monitor the effect of Ca^{2+}-induced conformational changes between the protein-protein interactions of calmodulin and M13 (Abstract). CFP was used as the donor and YFP was used as the acceptor (Figure 1a-c; page 885, right column, first full paragraph; page 886, right column, first full paragraph). In addition, Miyawaki et al. teach that, as expected, Ca^{2+}, acts as an agonist by inducing a conformational change in the protein-protein interaction of calmodulin and M13 (Figure 1a) as detected by an increased efficiency of FRET in response to increasing Ca^{2+} concentrations (Figure 2a).

To summarize, White et al. teach that GPCRs can oligomerize by forming either homodimers (i.e. same regions) or heterodimers (i.e. different regions). This protein-protein interaction taught by White et al. is also taught by Miyawaki et al. since Miyawaki et al. teach that heterodimerization of calmodulin and M13 proteins occurs. Miyawaki et al. teach that this dimerization of calmodulin and M13 can be detected by FRET using calmodulin and M13 fusion proteins fused to fluorescent proteins including CFP and YFP. Therefore, given the teachings of oligomerization of the GPCRs of White et al. and the FRET assay to observe the oligomerization of the proteins of Miyawaki et al, it would have been obvious to one of ordinary skill in the art at the time of the present invention to have produced GPCR fusion proteins in which a first GPCR was fused to a fluorescence donor, such as CFP, and a second GPCR, which was either the same as (i.e. homologous) or different than (i.e. heterologous) said first GPCR, was fused to a fluorescence acceptor, such as YFP, for the purpose of detecting oligomerization of GPCRs using this

very specific and sensitive FRET assay. The only difference between the proteins of White et al. and those of Miyawaki et al. is that the proteins of Miyawaki et al. are not GPCRs. However, given that the oligomerization of calmodulin and M13 proteins can be detected using FRET, the artisan would have been motivated to produce GPCR-fluorescent fusion proteins to detect oligomerization using FRET since it was well-known at the time of the invention that GPCRs oligomerize (White et al.). In fact, Hebert et al., who teach that GPCRs oligomerize, even state that one of the next steps that is required in demonstrating that GPCRs for biologically regulated dimmers in whole cells is to use a real-time measurement assay such as fluorescence resonance energy transfer (FRET; page 8, last paragraph). Furthermore, given that Miyawaki et al. detected the oligomerization of two proteins using FRET, there would also have been a reasonable expectation of success for one of ordinary skill in the art at the time of the present invention to have detected the oligomerization of the two proteins of White et al. Furthermore, there would have been a reasonable expectation of success in producing the GPCR-fusion proteins of White et al. using the teachings of Miyawaki et al. since there is no difference in the recombinant techniques used to produce fusion proteins between either GPCRs and fluorescent proteins and non-GPCRs and fluorescent proteins.

In addition, it would have also have been obvious to one of ordinary skill in the art at the time of the invention to have determined whether a receptor agonist activated GPCR by enhancing or disrupting oligomerization since both Miyawaki et al. and White et al. teach that agonists affect the function of oligomers. Miyawaki et al. show that Ca^{2+} acts as an agonist for the calmodulin-M13 interaction by increasing FRET efficiency, as diagrammed in Figure 1a. Similarly, White et al. demonstrate that the known agonist, GABA, stimulates [^{35}S]GTPγS binding in cells expressing both R2 and R1 receptors. Therefore, the artisan would have been able to detect either an increased or decreased FRET efficiency compared to cells prior to the addition of an agonist since these would have been the proper basis control studies to have performed by one of ordinary skill in the art.

The artisan would have been motivated to have used a FRET assay in order to monitor the oligomerization of the GPCRs of White et al. since the use of FRET has several advantages over covalent labeling with fluorescent probes. First, it is a non-destructive spectroscopic method which combines the brightness of fluorescence indicators with the targeting ability of a biosynthetic indicator in which the indicator is generated *in situ* by gene transfer into the cells or organism, obviating the need for large-scale purification, labeling and microinjection of recombinant proteins that must be soluble. Second, the sites of the fusions are exactly defined, providing a homogenous product. Third, the chromophore is fixed into the protein, as opposed to the use of a flexible linker which partly decouples the fluorophore orientation from

the protein to which it is attached (Miyawaki et al. page 883, top of left column). Claims 5 and 10 are rejected since they depend from rejected claims 1 and 6, respectively.

B. Claims 5 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over White et al. (reference AG on the IDS of Paper No.3) in view of Miyawaki et al. (Nature 388:882-887, 1997), further in view of Gama et al. (J. Biol. Chem. 273:29712-29718, 1998) and further in view of Hebert et al. (reference AI on the IDS of Paper No. 3).

Claims 5 and 10 depend from claims 1 and 6, respectively. Claim 1 recites a method of detecting oligomerization of GPCRs by transfecting a cell with a GPCR fused to a fluorescence donor and a GPCR fused to a fluorescence acceptor, exciting the fluorescence donor and detecting FRET such that if the FRET emission is greater that the FRET emission in control cells expressing only the acceptor, the GPCR oligomerization is detected. Claim 6 recites a method of determining whether an agonist enhances or disrupts GPCR oligomerization by transfecting a cell with a GPCR fused to a fluorescence donor and a GPCR fused to a fluorescence acceptor, exciting the fluorescence donor and detecting FRET such that , if the efficiency of FRET detected in cells contacted with an agonist is *greater* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by enhancing oligomerization. Conversely, if the efficiency of FRET detected in cells contacted with an agonist is *less* than that detected in cells prior to contact with said agonist, then receptor activation has occurred by disrupting oligomerization. Claims 5 and 10 recite that, in their respective methods, a GPCR region is truncated.

The teachings of both White et al. and Miyawaki et al. are discussed in the above (paragraph A) rejection of claims 1-10 under 35 USC 103(a). Neither White et al., nor Miyawaki et al. teach a method of detecting oligomerization of truncated G protein-coupled receptors using FRET, or a method of determining whether a receptor agonists activates GPCRs by enhancing or disrupting oligomerization of the truncated GPCRs of claims 5 and 10 using FRET. However, Gama et al. do teach that calcium sensing receptors (CaRs), which are GPCRs, can be truncated. In addition, Gama et al. produced fusion proteins comprising these truncated CaRs by combining the truncated CaRs with green fluorescent proteins (GFP) in order to assess any alterations in Ca^{2+}-dependent activation or desensitization (Abstract). Gama et al. not only demonstrate that truncated CaRs fused with GFP are functional (Table I, on page 29716), but that GFP can be used to visualize the location and movement of these truncation-mutant fusion proteins (Figure 4).

It would have been obvious to one of ordinary skill in the art at the time of the present invention to have produced the truncated GPCR of Gama et al. and to have coupled this protein to a fluorescent fusion protein for use in a FRET assay since, not only have fluorescent fusion proteins comprising GPCRs (i.e. CaR) been produced, but Gama et al. also teach that CaRs represent a novel member of a GPCR family which includes metabotropic glutamate receptors (mGluRs; page 29712, right column, first full paragraph). As stated in the previous rejection under 35 USC 103(a), White et al. teach that homodimerization has been reported for several seven transmembrane receptors (i.e. GPCRs), including metabotropic glutamate receptors (page 682, left column, first paragraph). In fact, Gama et al. teach that CaRs are present as disulfide-linked dimers in membranes (page 29716, right column).

The only differences between the proteins of Gama et al. and those of Miyawaki et al. are that the proteins of Miyawaki et al. are not GPCRs, nor are they truncated. However, given that the oligomerization of calmodulin and M13 proteins can be detected using FRET, the artisan would have been motivated to produce GPCR-fluorescent fusion proteins to detect oligomerization using FRET since it was well-known at the time of the invention that GPCRs oligomerize (White et al.). In fact, Hebert et al., who teach that GPCRs oligomerize, even state that one of the next steps that is required in demonstrating that GPCRs for biologically regulated dimmers in whole cells is to use a real-time measurement assay such as fluorescence resonance energy transfer (FRET; page 8, last paragraph). Furthermore, given that Miyawaki et al. detected the oligomerization of two proteins using FRET, there would also have been a reasonable expectation of success for one of ordinary skill in the art at the time of the present invention to have detected the oligomerization of the two proteins of Gama et al. Furthermore, there would have been a reasonable expectation of success in producing the GPCR-fusion proteins of Gama et al. using the teachings of Miyawaki et al. since there is no difference in the recombinant techniques used to produce fusion proteins between either GPCRs (e.g. CaR) and fluorescent proteins and non-GPCRs (e.g. calmodulin and M13) and fluorescent proteins.

Furthermore, one of ordinary skill in the art would have been motivated to have produced truncated CaR-fluorescent fusion proteins, as taught by Gama et al., to be used in the FRET assay of Miyawaki et al. since Gama et al. teach that studies on mGluRs suggest that carboxyl-terminal differences affect receptor-mediated signaling as well as agonist-independent activity (page 29712, right column, last paragraph) and that these truncation mutants which retain activity but have variable deletions of potential protein-protein interaction domains will make hunting for regulatory protein partners straightforward (Gama et al. page 29718, second-to-last paragraph). In the last paragraph of this page, Gama et al. also teach that the availability of GFP-tagged receptor truncations makes further exploration of the role of the

carboxy-terminal intracellular domain feasible and may generate paradigms generalizable to the GPCR superfamily.

Therefore, the artisan would have been motivated to have used a FRET assay in order to monitor the oligomerization of the GPCRs of Gama et al. since the use of FRET has several advantages over covalent labeling with fluorescent probes. First, it is a non-destructive spectroscopic method which combines the brightness of fluorescence indicators with the targeting ability of a biosynthetic indicator in which the indicator is generated *in situ* by gene transfer into the cells or organism, obviating the need for large-scale purification, labeling and microinjection of recombinant proteins that must be soluble. Second, the sites of the fusions are exactly defined, providing a homogenous product. Third, the chromophore is fixed into the protein, as opposed to the use of a flexible linker which partly decouples the fluorophore orientation from the protein to which it is attached (Miyawaki et al. page 883, top of left column).

7. Conclusion

A. No claim is allowed.

Advisory information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Robert Landsman whose telephone number is (703) 306-3407. The examiner can normally be reached on Monday - Friday from 8:00 AM to 5:00 PM (Eastern time) and alternate Fridays from 8:00 AM to 5:00 PM (Eastern time).

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Gary Kunz, can be reached on (703) 308-4623.

Official papers filed by fax should be directed to (703) 308-4242. Fax draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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